

Cardiomyopathy syndrome (CMS): Cardiac pathology and cause in field material.

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Overview

- Introduction & Project description
- Material and methods
- Results
- Conclusions
- Future work

Introduction: short description CMS

- First described Norway 1985 (Scotland 2000)
- Farmed adult Atlantic salmon in seawater
- Enigmatic syndrome: "hjertesprekk"
- Løvoll *et al* (2010): pyrosequencing
- Haugland *et al* (2011): cultured and identified novel **totivirus** (PMCV)



Project description

Cardiomyopathy syndrome: A multi-task approach to reduce losses and improve knowledge (2008-2011).

Our part: Epidemiology and pathogenesis

Objectives: Risks factors associated CMS (pathology)
Diagnostic criteria for CMS

Project no. 187301/S40:

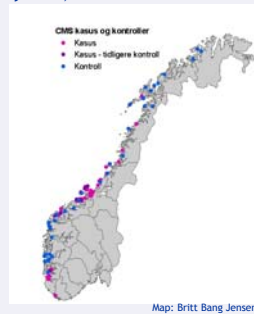


Materials and methods:

Case-control (April 2009-May 2010)

86 Norwegian farms

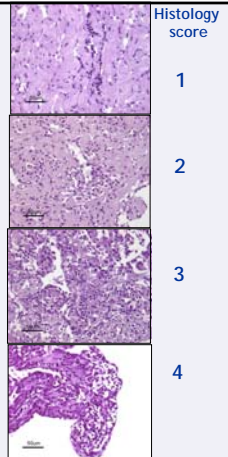
- CASES: CMS diagnosed (NVI) before sampling
- CONTROLS: NO CMS before sampling.
- CONTROLS developed CMS after sampling.



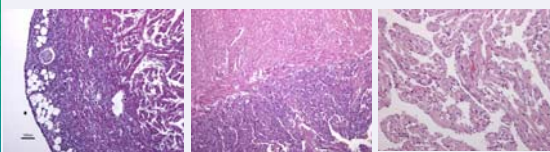
Histopathology

- 10-30 fish per farm (blind)
- 2300 hearts
- The atrium, spongy and compact layers of the ventricle and epicardium were graded from 0 to 4
- Histology all organs of some fish per farm to confirm the diagnosis.

Individual fish diagnosis & farm diagnosis



Differential diagnoses: The heart is a central organ in the organism and plays an important role in the development of several diseases.



2317 hearts classified as: CMS, HSMI, PD
No Lesions Observed (NLO)
Myocarditis, mild myocarditis
Mixed cases (CMS and/or HSMI; CMS and /or PD)
Other Cardiac Lesions (OCL)

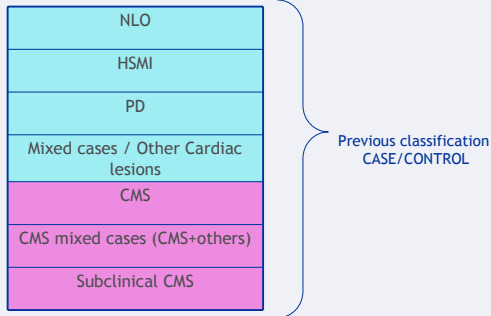
RT-PCR: confirmatory tool

- One-step real time RT-PCR: PMCV 500 fish (65 farms)

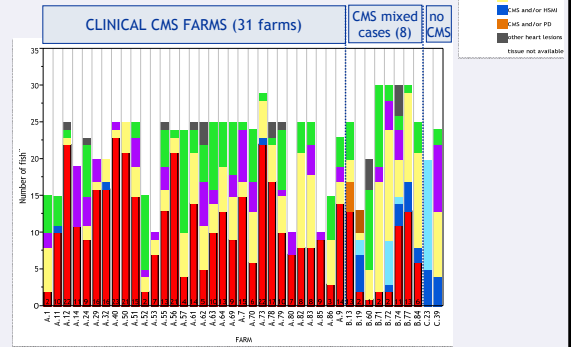
Ct-levels

■	Ct < 25 (high viral load)
■	Ct 25-30 (mid viral load)
■	Ct 30-40 (low viral load)
■	No Ct / Ct > 40 (neg)

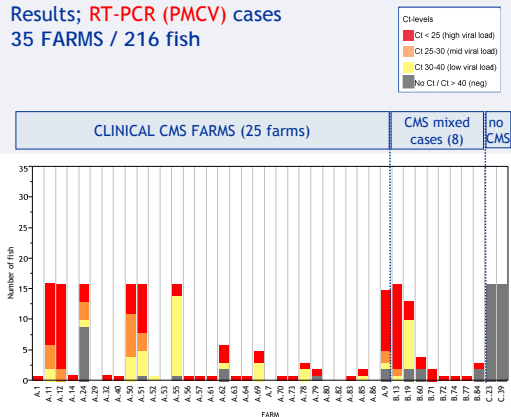
Histopathology + RT-PCR: FARM DIAGNOSIS 86 farms (89 samplings)



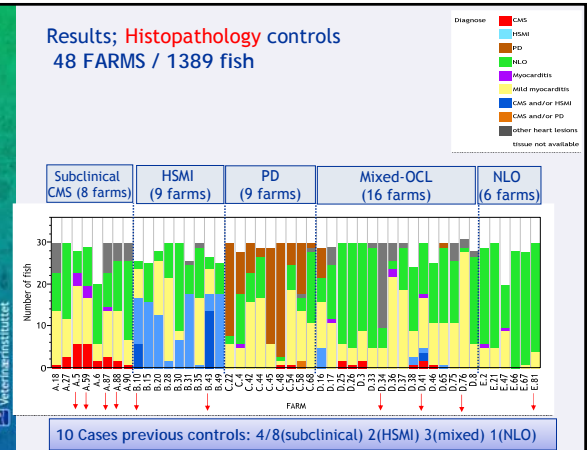
Results; Histopathology cases 41 FARMS / 927 fish



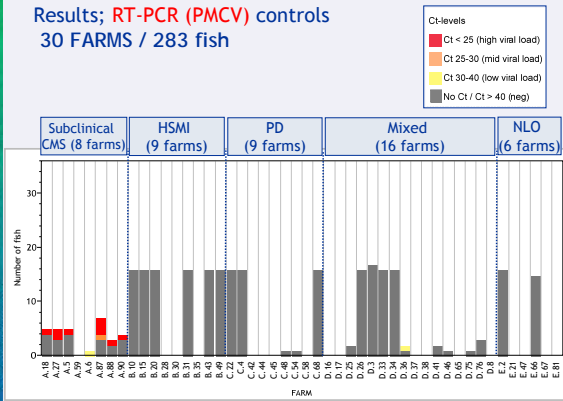
Results; RT-PCR (PMCV) cases 35 FARMS / 216 fish



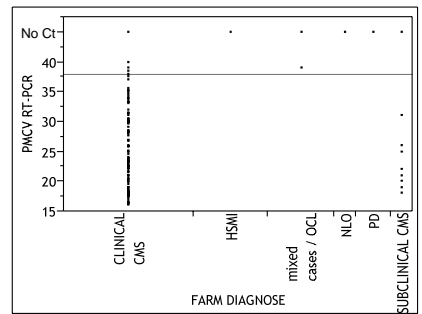
Results; Histopathology controls 48 FARMS / 1389 fish



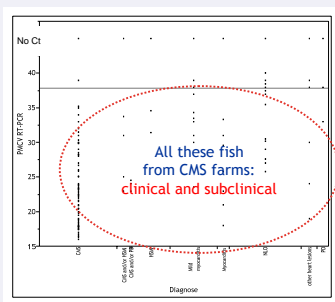
Results; RT-PCR (PMCV) controls 30 FARMS / 283 fish



Results; Relation PMCV (RT-PCR) and farm classification (500 fish, 65 farms)

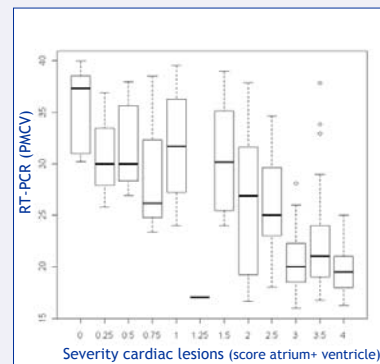


Results; Relation PMCV (RT-PCR) and individual fish HP diagnose (500 fish, 65 farms)

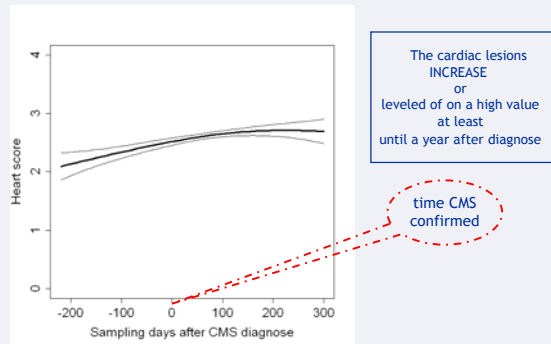


- PMCV in most CMS fish
- Also PMCV in fish
 - NLO
 - mild myocarditis
 - myocarditis
- But PMCV in few
 - HSMI
 - PD
 - mixed
 - other cardiac lesions

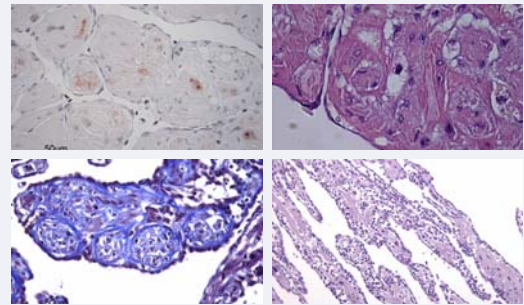
Results: Relation severity lesions with viral load (148 PMCV positive fish, 34 farms)



Results: Severity of cardiac lesions related with the time after confirmation of CMS (49 farms, 1164 fish)



Results: CMS cases (broodstock) >500 days after first CMS confirmation



Conclusions

- Diagnosis: histopathology not enough in mixed cases and subclinical CMS.
 - combination HP and PCR more specific diagnosis
- Disease is underdiagnosed: 4/8 subclinical cases not diagnosed.
- Virus and CMS lesions detectable much earlier than disease (4 months earlier)
- Viral persistence (detected after 1,5 year)

Future work

- Pathogenesis:
 - how the virus infect the fish?
 - how the virus spread in the fish and which organs are infected?
 - RT-PCR and ISH in different organs of field cases
 - what kills the fish?
 - No mortality in challenge trials
 - No problems in young salmon
 - Genetic variation within PMCV?
 - Sequencing: 50 fish from clinical cases are being sequenced.
 - Possibility to sequence fish from subclinical cases.
 - Possibility to validate IHCM (when PMCV Antibody available) in field material
 - Gen bank: all organs from 2300 fish (86 farms) in formalin fixed, paraffine blocks and RNA-later.
- Plenty of material and still a lot to learn but lack of funding !

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